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EDITORIAL

EGF receptor activation by G-protein coupled receptors

One of the central tenets of cell activation by growth factors and hormones has been that a ligand binds specifically to its cognate receptor, and activates receptor-dependent binding and specific cellular responses. However, recent studies have determined that this paradigm does not adequately explain the intracellular signaling pathways activated by the binding of an individual ligand to its receptor. Increasing evidence has suggested that activation of one class of receptors can lead to transactivation of a different class of receptors that eventuates in signal transduction pathways. Specifically, ligand binding to a number of G-protein coupled receptors (GPCRs) can induce transactivation of the epidermal growth factor (EGF) receptor, which mediates tyrosine kinase cascades of intracellular signaling.

The EGF receptor is a classic example of the receptor tyrosine kinase (RTK) class, which mediates a broad range of growth factor-dependent physiologic cellular responses, including proliferation, differentiation, survival and motility. A family of structurally related proteins, including EGF, transforming growth factor-α (TGF-α), amphiregulin, heparin-binding EGF (HB-EGF), betacellulin and epiregulin, serve as agonists for the EGF receptor. Activation of the intrinsic tyrosine kinase of the EGF receptor in response to ligand binding results in phosphorylation of a subset of the receptor's tyrosine residues that create binding sites for Src homology 2 (SH2) and phosphotyrosine binding (PTB) domain-containing proteins. The molecules that are recruited to these sites represent either enzymes that are activated as a result of tyrosine phosphorylation, such as Src, phospholipase Cγ (PLCγ) and phosphatidyl-inositol-3 kinase (PI3 kinase) or adaptor molecules (SHC) that activate downstream signaling pathways, such as MAP kinase pathways, as a result of formation and receptor association of a Shc-Grb2-SOS complex and induction of Raf function.

Tyrosine kinase-mediated signaling pathways have long been recognized to mediate the cellular responses resulting from ligand binding to RTKs such as the EGF receptor. Subsequently, it was discovered that receptor activation of receptors without intrinsic tyrosine kinase activity could also induce tyrosine kinase cascades. An example of such a signaling pathway is interferon alpha receptor activation of non-receptor-mediated tyrosine kinases of the Janus kinase (Jak) family that then phosphorylate substrate proteins called STATs (signal transducers and activators of transcription). The phosphorylated STAT proteins move to the nucleus, bind specific DNA elements, and direct transcription [1].

More recently, ligand binding to a number of GPCRs has also been shown to result in tyrosine kinase cascades. GPCRs represent the largest group of cell-surface receptors and consist of seven transmembrane domains with an extracellular amino terminus and an intracellular carboxy terminus. Ligand binding results in binding, activation and dissociation of heterotrimeric G proteins, with initiation of intracellular signaling response by G_{α} and $G_{\beta\gamma}$ subunits. The nature of the signaling responses is largely dependent upon the identity of the α subunit of the heterotrimeric G proteins and includes modulation of adenylate cyclases, PLCβ and kinases. The determination that GPCR-mediated activation of ERK/MAP kinases was dependent upon tyrosine phosphorylation of SHC and formation of a SHC/Grb2 complex suggested that GPCR also activated tyrosine kinases. Subsequently, a number of GPCRs, including endothelin, adrenergic, angiotensin II, purogenic, muscarinic, LPA, thrombin and bombesin receptors, have been shown to induce tyrosine phosphorylation and association of signaling molecules to the EGF receptor. Either pharmacologic inhibition of tyrosine kinase activity or expression of dominant negative EGF receptors prevents the mitogenic responses elicited by GPCRs [2].

There is evidence that this GPCR-regulated transactivation of the EGF receptor may be mediated by activation of the non-receptor tyrosine kinase c-Src, or src-like tyrosine kinases [3]. Src is known to phosphorylate and associate with the EGF receptor, and pharmacologic inhibitors of Src, dominant negative Src constructs or overexpression of Csk, a tyrosine kinase that negatively regulates Src function, all inhibit GPCR transactivation of the EGF receptor. In this issue of *Kidney International*, Bokemeyer, Schmitz and Kramer demonstrate that in vascular smooth muscle, angiotensin II activates the EGF receptor through a c-Src dependent pathway, which results in activation of both Erks and PI-3 kinase. Angiotensin II-induced mitogenesis in these cells was inhibited by either EGF receptor-specific inhibitors or Src-specific tyrosine kinase inhibitors [4].

The mechanism(s) of activation of these tyrosine ki-

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nase cascades by GPCRs is an area of active investigation. There is indication that G_i and $G_{\beta\gamma}$ subunits activate Src family kinases, which then mediate EGF receptor phosphorylation without activation of the intrinsic receptor tyrosine kinase. In contrast, for G_q coupled receptors, EGF receptor tyrosine kinase inhibitors and receptor mutants prevent signal transduction, suggesting that receptor activation is necessary for signal transduction [3, 5]. Such a mechanism of action is consistent with the studies of Bokemeyer et al, since AT_1 receptors in vascular smooth muscle cells are coupled predominantly to G_q-mediated signaling pathways [6].

The activation of Src by certain GPCRs has been reported to be mediated through the activation of the cytoplasmic tyrosine kinase PYK2, which is activated by elevations of intracellular calcium. Presumably such a Ca^{2+}-mediated pathway may mediate EGF receptor transactivation in GPCR coupled to PLC activation, and PYK2 activation has been shown to be involved in EGF receptor transactivation by angiotensin II in vascular smooth muscle cells [7] and by bradykinin [8]. It is of interest that in these studies extracellular calcium was required for PYK2 activation.

An alternative mechanism by which GPCRs may activate Src kinases has also been described for β-adrenergic receptors, in which receptor phosphorylation by βark kinases leads to association of a β-arrestin-Src complex with the receptor and subsequent activation of the EGF receptor [5]. Finally, certain cellular stresses, such as osmotic shock, free radicals and UV irradiation, induce EGF receptor transactivation by interfering with phosphotyrosine phosphatases [9], although such a mechanism has not yet been invoked for GPCR-mediated transactivation.

Although this developing paradigm of GPCR-transactivation of EGF receptors posits that the process occurs through ligand-independent EGF receptor activation, a recent study has suggested a different mechanism. The known ligands for the EGF receptor, such as HB-EGF, exist as transmembrane integral proteins that are cleaved by protein kinase C- and calcium-activated metalloproteinases to release the soluble growth factors [10]. Ullrich and associates have recently demonstrated that activation of a variety of GPCRs, including muscarinic, bombesin, thrombin, endothelin and LPA receptors, induced the processing of proHB-EGF to its soluble form, and metalloproteinase inhibitors and HB-EGF inhibitors prevented GPCR-dependent EGF receptor transactivation and tyrosine phosphorylation of Shc and the multidocking protein, Gab1 [11]. Therefore, an alternative or additional means for EGF receptor transactivation may be through secondary autocrine activation, in which GPCR activation induces metalloproteinase-dependent cleavage of EGF receptor ligands, which then activate the EGF receptor and initiate EGF receptor-dependent signaling pathways.

In summary, these exciting discoveries concerning GPCR-dependent EGF receptor transactivation have taught us that the perceived dichotomy between GPCR-mediated- and RTK-mediated-signaling pathways may be false and that cell activation by different classes of agonists initiates crosstalk that results in utilization of common signaling pathways.

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